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(54) Title: DNA IMMUNIZATION AGAINST *CHLAMYDIA* INFECTION

(57) Abstract

Nucleic acid, including DNA, immunization to generate a protective immune response in a host, including humans, to a major outer membrane protein of a strain of *Chlamydia*, preferably contains a nucleotide sequence encoding a MOMP or a MOMP fragment that generates antibodies that specifically react with MOMP and a promoter sequence operatively coupled to the first nucleotide sequence for expression of the MOMP in the host. The non-replicating vector may be formulated with a pharmaceutically-acceptable carrier for *in vivo* administration to the host.

TITLE OF INVENTION

DNA IMMUNIZATION AGAINST *CHLAYMDIA* INFECTION

FIELD OF INVENTION

The present invention relates to immunology and, in particular, to immunization of hosts using nucleic acid to provide protection against infection by *chlaymdia*.

BACKGROUND OF THE INVENTION

DNA immunization is an approach for generating protective immunity against infectious diseases (ref. 1 - throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosure of these references are hereby incorporated by reference into the present disclosure). Unlike protein or peptide based subunit vaccines, DNA immunization provides protective immunity through expression of foreign proteins by host cells, thus allowing the presentation of antigen to the immune system in a manner more analogous to that which occurs during infection with viruses or intracellular pathogens (ref. 2). Although considerable interest has been generated by this technique, successful immunity has been most consistently induced by DNA immunization for viral diseases (ref. 3). Results have been more variable with non-viral pathogens which may reflect differences in the nature of the pathogens, in the immunizing antigens chosen, and in the routes of immunization (ref. 4). Further development of DNA vaccination will depend on elucidating the underlying immunological mechanisms and broadening its application to other infectious diseases for which existing strategies of vaccine development have failed.

Chlamydia trachomatis is an obligate intracellular bacterial pathogen which usually remains localized to

a transcriptional promoter,

a DNA molecule encoding a *C. trachomatis* MOMP polypeptide comprising a MOMP polynucleotide at least 27 base pairs in length from a sequence provided in Appendix A thereto, and

a transcriptional terminator, wherein at least one of the transcriptional regulatory elements is not derived from *Chlamydia trachomatis*. There is no disclosure or suggestion in this prior art to effect DNA immunization with any such constructs.

WO 94/26900 describes the provision of hybrid picornaviruses which express chlamydial epitopes from MOMP of *Chlamydia trachomatis* and which is capable of inducing antibodies immuno-reactive with at least three different *Chlamydia* serovars. The hybrid picornavirus preferably is a hybrid polio virus which is attenuated for human administration.

SUMMARY OF THE INVENTION

The present invention is concerned with nucleic acid immunization, specifically DNA immunization, to generate in a host protective antibodies to a MOMP of a strain of *Chlamydia*. DNA immunization induces a broad spectrum of immune responses including Th1-like CD4 responses and mucosal immunity.

Accordingly, in one aspect, the present invention provides an immunogenic composition *in vivo* for *in vivo* administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of *Chlamydia*, comprising a non-replicating vector comprising a nucleotide sequence encoding a MOMP or MOMP fragment that generates a MOMP-specific immune response, and a promoter sequence operatively coupled to said nucleotide sequence for expression of said MOMP in the host; and a pharmaceutically-acceptable carrier therefor.

sequence and into which the nucleotide sequence is inserted in operative relation to the promoter sequence.

In the additional aspect of the invention, a further aspect of the present invention provides a method of producing a vaccine for protection of a host against disease caused by infection with a strain of *Chlamydia*, which comprises isolating a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or a MOMP fragment that generates a MOMP-specific immune response, operatively linking said nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said MOMP when introduced to a host to produce an immune response to said MOMP, and formulating said vector as a vaccine for *in vivo* administration to a host.

Advantages of the present invention, therefore, include a method of obtaining a protective immune response to infection carried by a strain of *Chlamydia* by DNA immunization of DNA encoding the major outer membrane protein of a strain of *Chlamydia*.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 illustrates delayed-type hypersensitively (DTH) responses following immunization. DTH responses following DNA vaccination. Balb/c mice (four per group) were immunized intramuscularly (pMOMP IM) or intranasally (pMOMP IN) with plasmid DNA containing the coding sequence of the MoPn MOMP gene or with MoPn elementary bodies (EB) at 0,3,6 weeks. The control group was treated with the blank plasmid vector (pcDNA3). Fifteen days after the last immunization, mice were tested for MoPn-specific DTH response as follows: 25 µl of heat-inactivated MoPn EB (5×10^4 IFU) in SPG buffer was injected into the right hind footpad and the same volume of SPG buffer was injected into the

the blank plasmid vector (pcDNA3) at 0,3,6 weeks and pooled sera from each group were collected two weeks following the last immunization (day 10). The data represent mean ± SEM of the OD value of four duplicates.

Figure 5, comprising panels A and B, demonstrates that DNA vaccination with the MOMP gene enhanced clearance of MoPn infection in the lung. Groups of Balb/c mice were immunized with pMOMP (n = 10), pcDNA3 (n = 10) or saline (n = 5). Eighteen days after the last immunization, the mice were challenged intranasally with infectious MoPn (10^4 IFU). Panel A shows the body weight of the mice measured daily following challenge infection until the mice were sacrificed at day 10. Each point represents the mean ± SEM of the body weight change. * represents $\underline{P} < .05$ compared with pcDNA3 treated group. Panel B: the mice were sacrificed at day 10 postinfection and the MoPn growth in the lung was analyzed by quantitative tissue culture. The data represent mean ± SEM of the Log_{10}IFU per lung. * represents $\underline{P} < .01$ compared with pcDNA3 treated group.

Figure 6, comprising panels A and B, shows evaluation of the responses of mice to MoPn intranasal challenge infection. In Panel A, is shown change in body weight post challenge and in Panel B, is shown the growth of MoPn in lung tissue collected 10 days after challenge. Mice were sham immunized, immunized intraperitoneally with MoPn EBs, recovered from prior MoPn lung infection or immunized intramuscularly with pMOMP.

Figure 7 shows the elements and construction of plasmid pcDNA3/MOMP.

GENERAL DESCRIPTION OF THE INVENTION

To illustrate the present invention, plasmid DNA was constructed containing the MOMP gene from the *C.*

The data presented herein also demonstrate the importance in selection of an antigen gene for DNA immunization. The antigen gene elicits immune responses that are capable of stimulating recall immunity following exposure to the natural pathogen. In particular, injection of a DNA expression vector encoding the major surface protein (the pMOMP) but not one encoding a cytoplasmic enzyme (CTP synthetase) of *C. trachomatis* generated significant protective immunity to subsequent chlamydial challenge. The protective immune response appeared to be predominantly mediated by cellular immunity and not by humoral immunity since antibodies elicited by DNA vaccination did not bind to native EBs. In addition, MOMP DNA but not CTP synthetase DNA immunization elicited cellular immunity readily recalled by native EBs as shown by positive DTH reactions.

In addition, mucosal delivery of MOMP DNA is demonstrated herein to be significantly more efficient in inducing protective immunity to *C. trachomatis* infection than intramuscular injection. This may be relevant to the nature of *C. trachomatis* infection which is essentially restricted to mucosal surfaces and the efficiency of antigen presentation (ref. 14). The rich population and rapid recruitment of dendritic cells into the respiratory epithelium of the lung may be relevant to the enhanced efficacy of intranasal DNA immunization experiments (ref. 15). The data presented herein represents the demostration of a first subunit chlamydial vaccine which engenders substantial protective immunity.

Additionally, it may be possible to amplify (and/or canalize) the protective immune response by co-administration of DNAs that express immunoregulatory cytokines in addition to the antigen gene in order to

liposome (for example, as described in WO 9324640, ref. 12) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co- glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the MOMP and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 µg to about 1 mg of the MOMP gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen

be used to transfect skin, muscle, fat and mammary tissues of living animals.

2. **Immunoassays**

The MOMP genes and vectors of the present invention are useful as immunogens for the generation of anti-MOMP antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the MOMP. These MOMP specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution,

transcription under control of the human cytomegatovirus major intermediate early enhancer region (CMV promoter). The MOMP gene-encoding plasmid was transferred by electroporation into *E. coli* DH5αF which was grown in LB broth containing 100 μg/ml of ampicillin. The plasmids was extracted by Wizard™ Plus Maxiprep DNA purification system (Promega, Madison). The sequence of the recombinant MOMP gene was verified by PCR direct sequence analysis, as described (ref. 20). Purified plasmid DNA was dissolved in saline at a concentration of 1 mg/ml. The DNA concentration was determined by a DU-62 spectrophotometer (Beckman, Fullerton, CA) at 260 nm and the size of the plasmid was compared with DNA standards in ethidium bromide-stained agarose gel.

The MOMP gene containing plasmid, pcDNA3/MOMP is illustrated in Figure 7.

Example 2:

This Example illustrates DNA immunization of mice and the results of DTH testing.

A model of murine pneumonia induced by the *C. trachomatis* mouse pneumonitis strain [MoPn] was used (ref. 11). Unlike most strains of *C. trachomatis* which are restricted to producing infection and disease in humans, MoPn is a natural murine pathogen. It has previously been demonstrated that primary infection in this model induces strong protective immunity to reinfection. In addition, clearance of infection is related to CD4 Th1 lymphocyte responses and is dependent on MHC class II antigen presentation (ref. 11).

For experimental design, groups of 4 to 5 week old female Balb/c mice (5 to 13 per group) were immunized intramuscularly (IM) or intranasally (IN) with plasmid DNA containing the coding sequence of the MoPn MOMP gene (1095 bp), prepared as described in Example 1, or with

Example 3:

This Example illustrates DNA immunization of mice and the generation of antibodies.

Injection of CTP synthetase DNA as described in Example 2 resulted in the production of serum antibodies to recombinant CTP synthetase (Table 1) (ref. 14). Antigen-specific serum Abs were measured by ELISA. Flat-bottom 96-well plates (Corning 25805, Corning Science Products, Corning, NY) were coated with either recombinant chlamydial CTP-synthetase (1 µg/ml) or purified MoPn EBs (6 x 10^4 IFU/well) overnight at 4°C. The Plates were rinsed with distilled water and blocked with 4% BSA PBS-Tween and 1% low fat skim milk for 2 hours at room temperature. Dilutions of sera samples were performed in 96-well round bottom plates immediately prior to application on the antigen coated plates. The plates were incubated overnight at 4°C and washed ten times. Biotinylated goat anti-mouse IgG1 or goat anti-mouse IgG2a (Southern Biotechnology Associates, Inc. Birmingham, AL) were next applied for 1 hour at 37°C. After washing, streptoavidin-alkaline phosphatase conjugate (Jackson ImmunoResearch Laboratories, Inc. Mississagua, Ontario, Canada) were added and incubated at 37°C for 30 min. Following another wash step, phosphatase substrate in phosphatase buffer (pH 9.8) was added and allowed to develop for 1 hour. Th plates were read at 405 nm on a BIORAD 3550 microplate reader.

lgG2a antibody titers were approximately 10-fold higher than lgG1 antibody titers suggesting that DNA immunization elicited a more dominant T_{H1}-like response. Injection of MOMP DNA as described in Example 2 resulted in the production of serum antibodies to MOMP (Table 2) as detected in an immunoblot assay (Figure 2). However,

(log_{10} IFU 1.3 ± 0.3; mean $\pm$ SEM) than those of control mice immunized with the blank vector (log_{10} IFU 5.0 ± 0.3; $p<0.01$) (see Figure 2, Panel B). Mice intramuscularly immunized with MOMP DNA had chlamydial lung titers that were more than 10-fold lower than the unmodified vector group ($p = 0.01$). Mice intranasally immunized with MOMP DNA had significantly lower chlamydial lung titers than mice immunized with MOMP DNA intramuscularly (log_{10} IFU 1.3 ± 0.8 versus log_{10} IFU 0.66 ± 0.3 respectively; $p = 0.38$). The substantial difference (2.4 logs) in chlamydial lung titers observed between the intranasally and intramuscularly MOMP DNA immunized mice suggests that mucosal immunization is more efficient at inducing immune responses to accelerate chlamydial clearance in the lung. The lack of protective effect with the unmodified vector control confirms that DNA *per se* was not responsible for the immune response. Moreover, the absence of protective immunity following immunization with CTP synthetase DNA confirms that the immunity was specific to the MOMP DNA (see Table 1). Figure 5 shows similar challenge data at a higher challenge dose.

Example 5:

This Example describes the construction of p½MOMP.

A PCR cloned MoPn gene was constructed containing a deletion mutation in codon 177. This recitation yields a truncated MOMP protein containing approximately 183 amino-terminal amino acids (ref. 10). This construct, termed p½MOMP, was cloned into the vector pcDNA3 (Invitrogen), in the manner described in Example 1.

Example 6:

This Example illustrates immunization of mice with p½MOMP.

was elicited suggesting that protective sites can be found in the amino terminal half of the protein.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by strain of *Chlamydia*, specifically *C. trachomatis*, employing a non-replicating vector, specifically a plasmid vector, containing a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* and a promoter to effect expression of MOMP in the host. Modifications are possible within the scope of this invention.

Table 2 Serum antibody Elisa titers to *Chlamydia trachomatis* mouse pneumonitis recombinant MOMP and Ebs were measured 60 days after the initial immunization among mice immunized with blank vector alone (pcDNA3), vector containing the MOMP gene (pMOMP) and vector containing the CTP synthetase gene (pCTP). Non-immunized mice were also tested.

	rMOMP		EB	
	IgG2a	IgG1	IgG2a	IgG1
pcDNA3	<2.6*	<2.6	<2.6	<2.6
pMOMP	3.77±0.1	2.90±0.14	3.35±0.11	<2.6
pCTP	ND	ND	<2.6	<2.6
Preimmunization	<2.6	<2.6	<2.6	<2.6

* $\log_{10}$ mean ± SE IgG isotype specific antibody titer

ND = not done

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CLAIMS

What we claim is:

1. An immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of *Chlamydia*, comprising a non-replicating vector comprising:

a nucleotide sequence encoding a MOMP or MOMP fragment that generates a MOMP-specific immune response, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said MOMP in the host; and

a pharmaceutically-acceptable carrier therefor.

2. The composition of claim 1 wherein said nucleotide sequence encodes a full-length MOMP.

3. The immunogenic composition of claim 1 wherein said nucleotide sequence encodes an N-terminal fragment of the MOMP of approximately half the size of full-length MOMP.

4. The immunogenic composition of claim 1 wherein said promoter sequence is the cytomegalovirus promoter.

5. The immunogenic composition of claim 1 wherein said strain of *Chlamydia* is a strain producing chlamydial infections of the lung.

6. The immunogenic of claim 1 wherein said strain of *Chlamydia* is a strain of *chlamydia trachomatis*.

7. The immunogenic composition of claim 6 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

8. The composition of claim 1 wherein said immune response is predominantly a cellular immune response.

9. The composition of claim 1 wherein said nucleotide sequence encodes a MOMP which stimulates a recall immune

host.

21. A method of using a gene encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or MOMP fragment that generates a MOMP-specific immune response, to produce an immune response in a host, which comprises:

isolating said gene,

operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said MOMP when introduced into a host to produce an immune response to said MOMP, and

introducing said vector into a host.

22. The method of claim 21 wherein said gene encoding MOMP encodes a full length MOMP.

23. The method of claim 21 wherein said gene encoding MOMP encodes an N-terminal fragment of the MOMP of approximately half the size of full-length MOMP.

24. The method of claim 21 wherein said control sequence is the cytomegalovirus promoter.

25. The method of claim 21 wherein said strain of *Chlamydia* is a strain producing chlamydial infections of the lung.

26. The method of claim 21 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.

27. The method of claim 21 wherein said non-replicating vector comprises plasmid pcDNA3 containing said control sequence into which said gene encoding MOMP is inserted in operative relation to said control sequence.

28. The method of claim 21 wherein said immune response is predominantly a cellular immune response.

29. The method of claim 21 wherein said gene encodes a MOMP which stimulates a recall immune response following exposure to wild-type *Chlamydia*.

30. The method of claim 21 wherein said vector is introduced into said host intranasally.

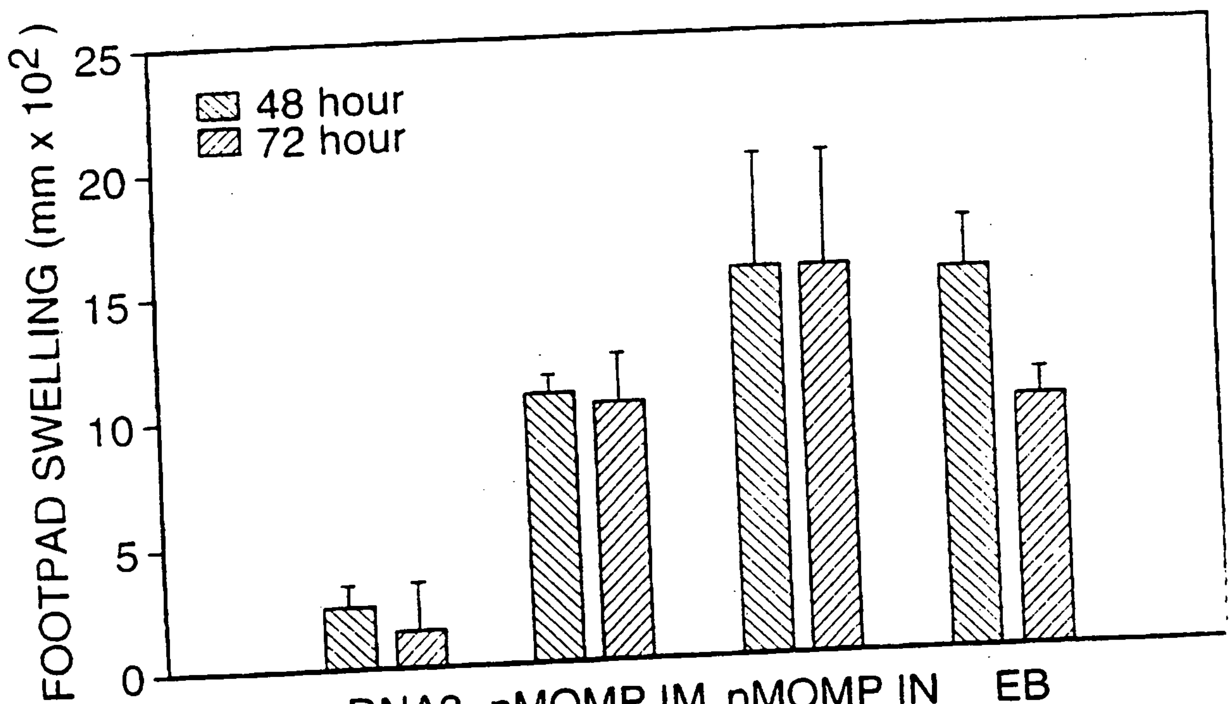

FOOTPAD SWELLING (mm x 10^2)
25
20
15
10
5
0
48 hour
72 hour
pcDNA3
pMOMP IM
pMOMP IN
EB

FIG.1

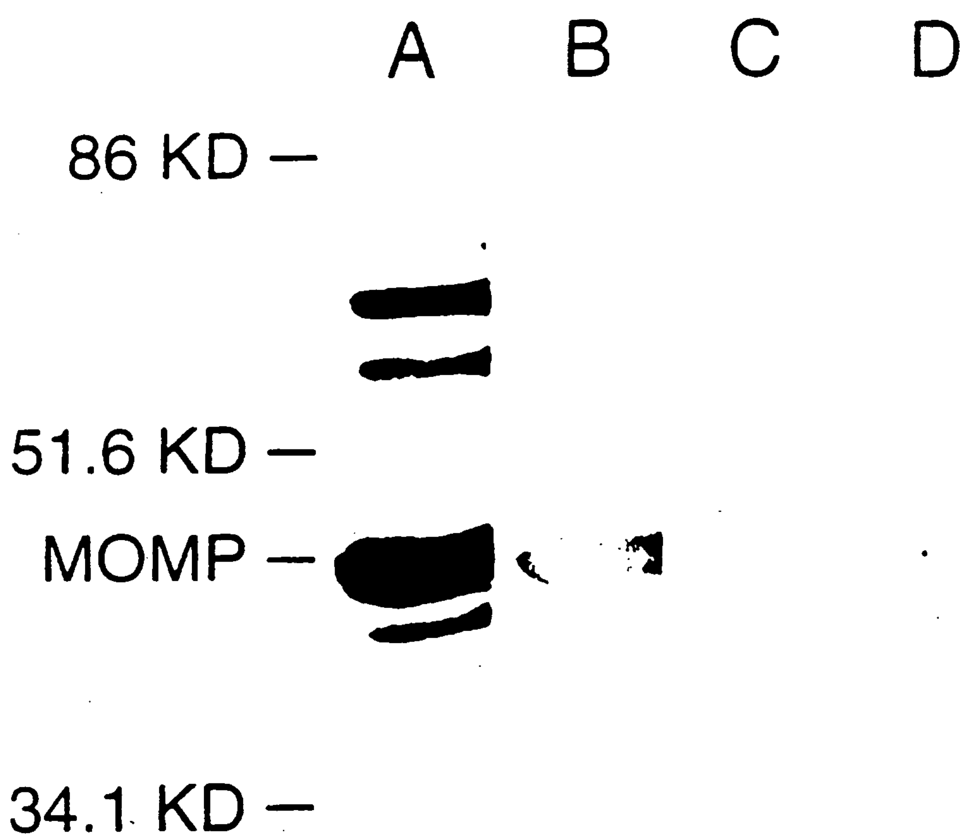


FIG.3

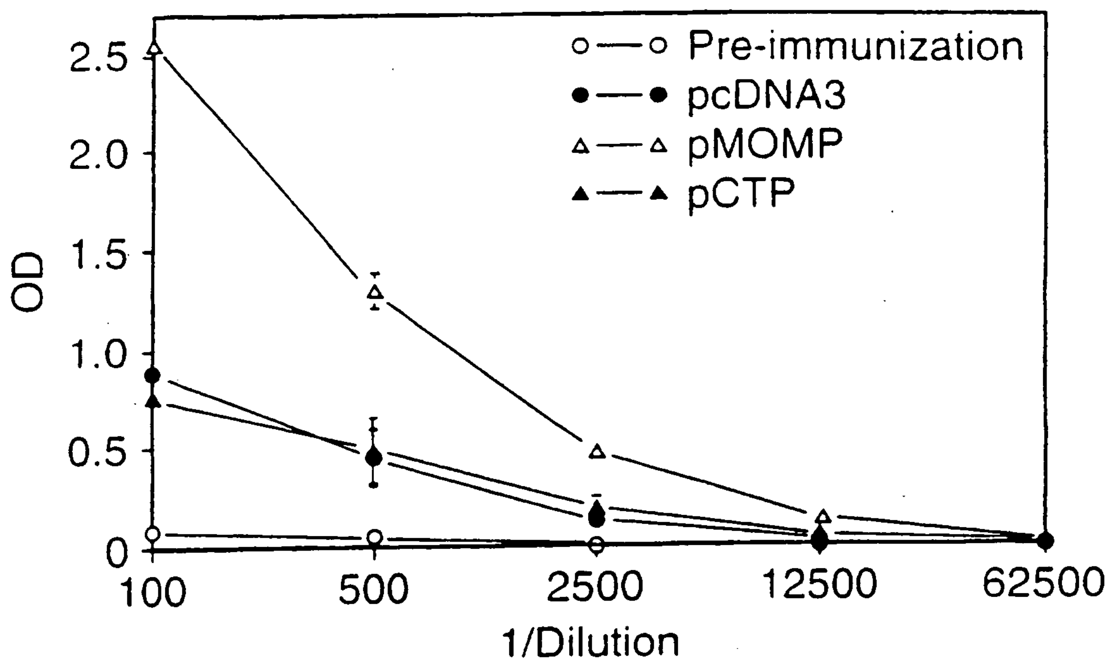

Pre-immunization
pcDNA3
pMOMP
pCTP
OD
2.5
2.0
1.5
1.0
0.5
0
100
500
2500
12500
62500
1/Dilution

FIG.4C

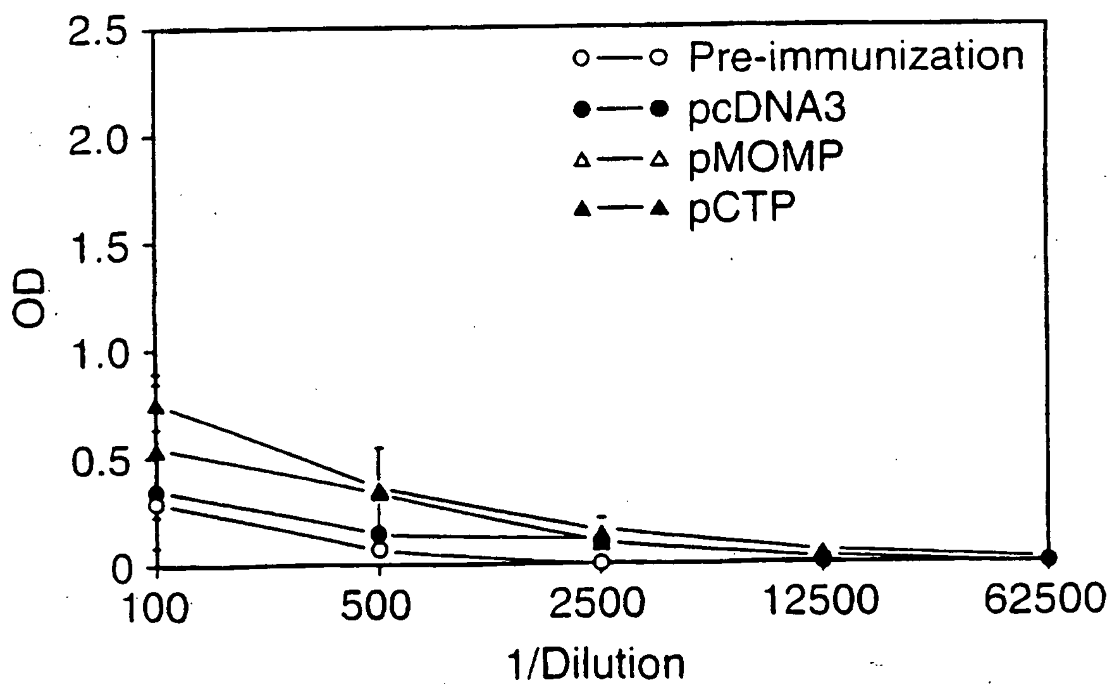
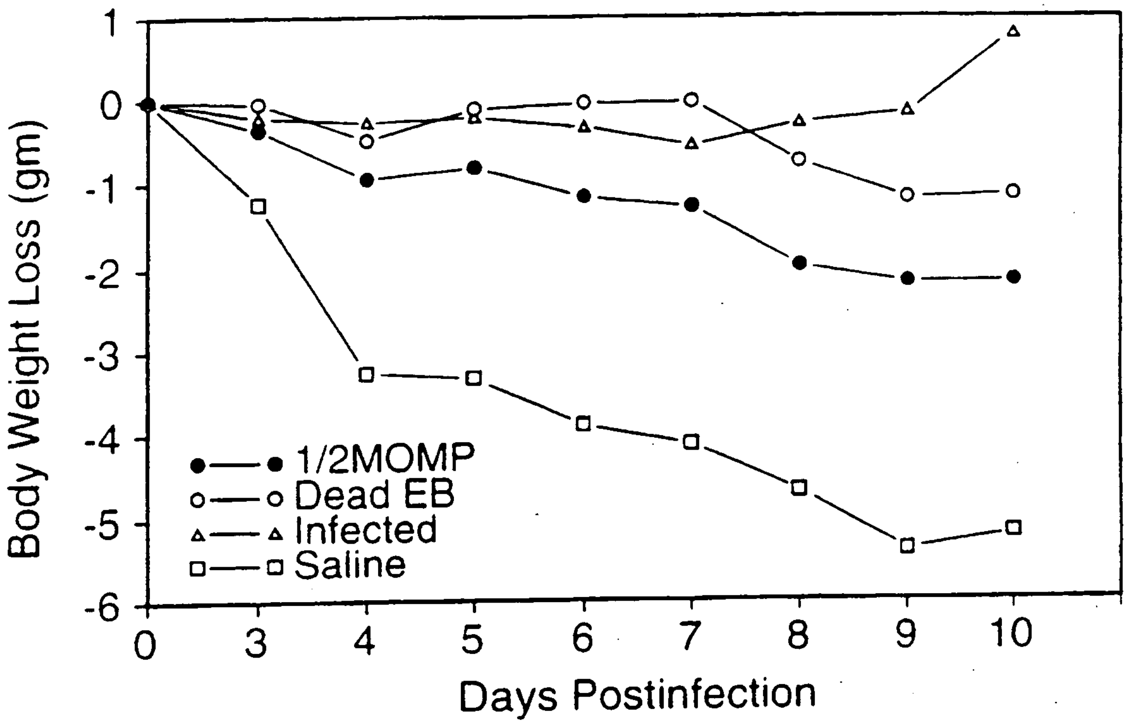
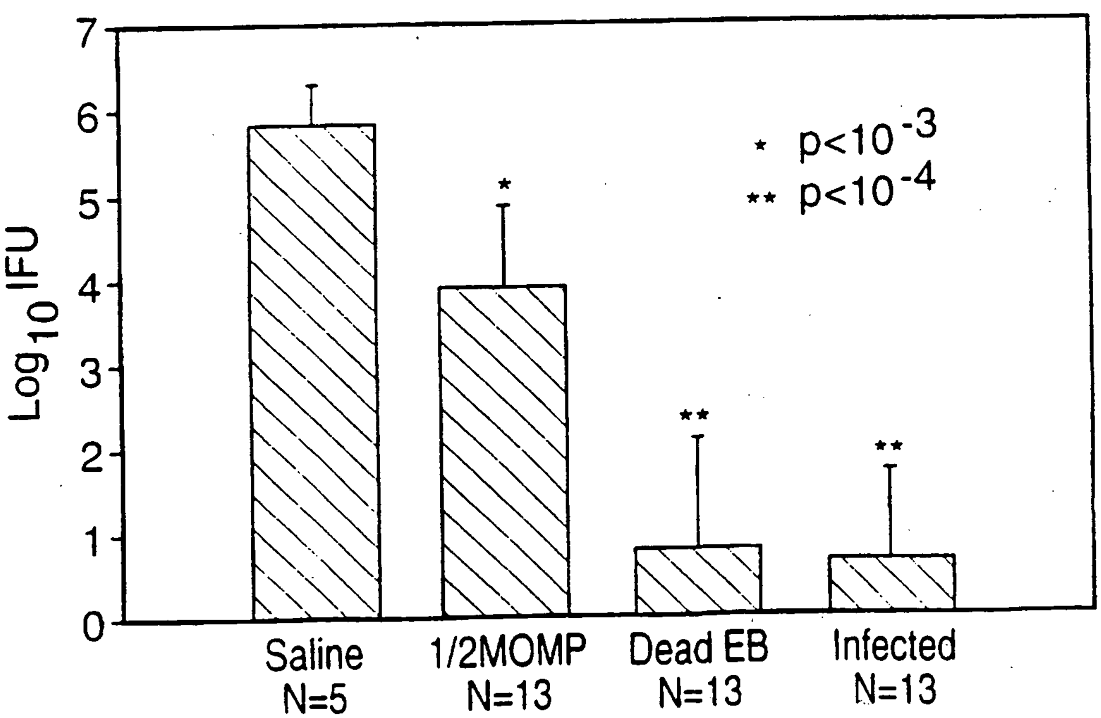

Pre-immunization
pcDNA3
pMOMP
pCTP
OD
2.5
2.0
1.5
1.0
0.5
0
100
500
2500
12500
62500
1/Dilution

FIG.4D



Body Weight Loss (gm)
1
0
-1
-2
-3
-4
-5
-6
1/2MOMP
Dead EB
Infected
Saline
0 3 4 5 6 7 8 9 10
Days Postinfection

FIG.6A



Log10IFU
7
6
5
4
3
2
1
0
* p<10-3
** p<10-4
*
**
**
Saline N=5
1/2MOMP N=13
Dead EB N=13
Infected N=13

FIG.6B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00500

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 A61K31/70 C07K14/295 //A61K39/118

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	EP 0 192 033 A (CHIRON CORPORATION) 27 August 1986 cited in the application see abstract and claims see page 7, line 33 - page 9, line 13 see page 11, line 8-33 see page 16, line 20 - page 18, line 22 ---	1-33
Y	DONNELLY ET AL.: "Protective efficacy of intramuscular immunization with naked DNA" ANNALS NEW YORK ACADEMY OF SCIENCES, vol. 772, 1995, pages 40-46, XP000576178 cited in the application see Introduction, Potential Clinical Uses and Conclusion --- -/--	1-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 1997	16.01.98

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 97/00500

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0192033 A	27-08-86	AT 143414 T	15-10-96
		DE 3650571 D	31-10-96
		DE 3650571 T	27-02-97